Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 145 (2007) 45–50
veterinary parasitology
www.elsevier.com/locate/vetpar

# Prevalence of *Toxoplasma gondii* in dogs from Colombia, South America and genetic characterization of *T. gondii* isolates

J.P. Dubey [a,*], J.A. Cortés-Vecino [b], J.J. Vargas-Duarte [b], N. Sundar [a], G.V. Velmurugan [a], L.M. Bandini [a], L.J. Polo [b], L. Zambrano [b], L.E. Mora [b], O.C.H. Kwok [a], T. Smith [c], C. Su [c]

[a] *United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA*
[b] *Laboratorio de Parasitología, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia-Sede Bogotá, Bogota, Colombia, South America*
[c] *Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845, USA*

Received 5 October 2006; received in revised form 21 November 2006; accepted 5 December 2006

**Abstract**

The prevalence of *Toxoplasma gondii* in 309 unwanted dogs from Bogotá, Colombia, South America was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT) and found in 52 (16.8%) of 309 dogs with titers of 1:20 in 20, 1:40 in six, 1:80 in 17, 1:160 in three, 1:320 in three, 1:1280 or higher in three. Some organs obtained after necropsy of dogs (hearts, tongues and brains, either separately or pooled) were used in bioassays carried out in mice (37 samples, of which 20 were assayed with separate organs and 17 were assayed with pooled organs), cats (pooled organs from six) and pooled organs of two dogs both in mice and cat. Mice receiving dog tissues were examined for *T. gondii* infection. Feces of cats that received dog tissues were examined for oocyst shedding. In total, *T. gondii* was isolated from tissues of 20 dogs (16 by bioassays in mice, 3 by bioassay in cats and 1 by bioassay in mice and cat). All infected mice from 7 of 17 isolates bioassayed in this host died of toxoplasmosis during primary infection. Only 10 of the 20 dogs whose tissues were bioassayed separately induced infections in mice. Interestingly, dog organs varied in their capacity to induce *T. gondii* infection in mice, hearts and tongues producing more positive results than the brain. The 20 *T. gondii* isolates obtained from seropositive dogs were PCR-RFLP genotyped using polymorphisms at 10 nuclear markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, a new SAG2 and an apicoplast marker Apico. Ten genotypes were revealed. These genotypes are different from the three predominant Types I, II and III lineages that are widely spread in North America and Europe. A new allele denoted u-3 at PK1 locus was identified in three isolates. This result supports previous findings that *T. gondii* population is highly diverse in Colombia.
Published by Elsevier B.V.

*Keywords:* *Toxoplasma gondii*; Dogs; Colombia; South America; Bioassays; Antibodies; Genotype

## 1. Introduction

*Toxoplasma gondii* infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked meat,

* Corresponding author. Tel.: +1 301 504 8128; fax: +1 301 504 9222.
*E-mail address:* jdubey@anri.barc.usda.gov (J.P. Dubey).

0304-4017/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetpar.2006.12.001

consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is unknown whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability or to other factors.

*T. gondii* isolates have been classified into three genetic Types (I–III) based on restriction fragment length polymorphism (RFLP) (Howe and Sibley, 1995; Howe et al., 1997) and until recently, *T gondii* was considered to be clonal with very little genetic variability. Based on newer markers for genetic characterization and using recently isolated strains, a higher genetic variability has been revealed than previously reported (Ajzenberg et al., 2004; Ferreira et al., 2006; Khan et al., 2006; Lehmann et al., 2006).

We have initiated a study of worldwide genetic diversity of *T. gondii* with ultimate objective to understand the protein variability among strains for immunoprophylaxis. In the present study, we attempted to isolate and characterize *T. gondii* from dogs from Bogotá, Columbia, South America. We also examined distribution of *T. gondii* in tissues of asymptomatic dogs to improve biological diagnosis.

## 2. Materials and methods

### 2.1. Naturally-infected dogs

Unwanted 309 dogs (148 males and 161 females, with 43 younger than 6 months, 15 between 6 month and 1 year old, 251 of 1 year and older) were killed between February and May 2006 (Table 1). The dogs were unclaimed pets or were caught by the municipality. They were euthanized by intravenous injection (Euthanex®, Invet, S.A. Bogotá, Colombia) by Centro Distrital de Zoonosis, Bogotá when efforts to place them in good homes had failed. At necropsy, brain, heart and blood samples were collected and kept at 4 °C until sent refrigerated by air to United States Department of Agriculture (USDA)'s Animal Parasitic Diseases Laboratory, Beltsville, MD, where all *T. gondii* bioassays were performed using protocols approved by the USDA. One to 3 days elapsed between killing of dogs and receipt of samples in Beltsville. The samples were received in excellent condition.

### 2.2. Serological examination

Sera of dogs were tested for *T. gondii* antibodies using two-fold serum dilutions from 1:20 to 1:1280 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

### 2.3. Bioassay of canine tissues for T. gondii infection

Tissues of all 43 seropositive dogs (11 with titers of 1:20, 6 with titers of 1:40 and 26 with titers of 1:80 or higher) were bioassayed in mice (35 dogs), in cats (6 dogs) and both mice and cats (2 dogs) (Table 1). Of the 37 seropositive dogs bioassayed in mice, brains, tongues and hearts of 20 dogs were each bioassayed individually and for the remainder 17 dogs all three tissues were pooled before bioassay in out-bred female Swiss Webster mice obtained from Taconic Farms, Germantown, New York, as described by Dubey et al. (2002). Each tissue (20–25 g) was homogenized individually, digested in acidic pepsin, neutralized and washed (Dubey, 1998); the homogenate (1 ml) inoculated subcutaneously into 3–5 mice (Table 2).

Brains, tongues and hearts from eight seropositive dogs (two with titers of 1:40, and six with titers of 1:80) were pooled and fed separately to eight *T. gondii*-free cats. Feces of cats were examined for shedding of *T.*

Table 1
Prevalence of *Toxoplasma gondii* in dogs from Bogotá, Colombia

| Experiment No. | Date killed | No. of dogs | Number of dogs seronegative (<1:20) | Number of dogs seropositive (≥1:20) | Number of dogs bioassayed | *T. gondii* isolated from dog tissues |
|---|---|---|---|---|---|---|
| 235 | 2-17-06 | 51 | 39 | 12 | 11[a] | 4 |
| 238 | 3-2-06 | 51 | 35 | 16 | 8 | 6 |
| 247 | 4-6-06 | 52 | 46 | 6 | 6 (6)[b] | 3 |
| 249 | 4-13-06 | 50 | 48 | 2 | 2 (2)[b] | 1 |
| 250 | 4-28-06 | 51 | 42 | 9 | 9 (9)[b] | 5 |
| 254 | 5-24-06 | 54 | 47 | 7 | 7 (3) [b] | 1 |
| Total | | 309 | 257 | 52 | 43 (20)[b] | 20 |

[a] Six dogs were bioassayed in cats, three were bioassayed in mice and two dogs were bioassayed both in cats and mice.
[b] Brains, hearts and tongues from these dogs were bioassayed separately in mice and were pooled from the remainder dogs.

Table 2
Isolation of *Toxoplasma gondii* from dogs from Bogotá, Colombia

| Dogs | | | Oocysts shed in cats (cat no.) | Isolation in mice[a] | | | Strain designation | Genotype[c] | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment no. and dog no. | MAT titer | Age/sex | | Brain | Heart | Tongue | | SAG1, SAG2, SAG3, BTUB, GRA6 | c22-8, c29-2, L358, PK1, new SAG2, Apico |
| Tx 235 | | | | | | | | | |
| 16 | 320 | 5 months/F | Yes (271) | Not done | | | TgDgCo1[d] | I,I,III,I,III | II,I,III,III,II,I |
| 19 | 80 | 6 months/F | Yes (243) | 1/4 Pooled tissues | | | TgDgCo2[d] | I,I,III,I,III | II,I,III,III,II,I |
| 39 | 80 | 6 years/F | Yes (258) | Not done | | | TgDgCo3[d] | I,I,III,I,III | II,I,III,III,I,I |
| 44 | 80 | 11 years/M | Yes (260) | Not done | | | TgDgCo4[d] | u-1,II,III,III,II | II,III,II,II,II,I |
| Tx238 | | | | | | | | | |
| 6 | 80 | 7 years/M | Not done | 1/5(22)[b] pooled tissues | | | TgDgCo5 | I,I,III,I,III | II,I,I,u-3,II,I |
| 9 | 80 | 5 months/F | Not done | 4/5 (16,17,20,36) Pooled tissues | | | TgDgCo6 | I(4),I (4),III (4),I (4),III (4) | II(2),I(2),I(2),u-3(2),II(1),I(2) |
| 14 | 80 | 5 years/M | Not done | 3/5(10,10,14) Pooled tissues | | | TgDgCo7 | I(3),III (3),III (3),III(3),III (3) | I (2),III (2),III (2),I (2),III (2),III (2) |
| 16 | 80 | 4 years/M | Not done | 1/5(24) pooled tissues | | | TgDgCo8 | I,III,III,I,III | II,I,III,III,III,I |
| 26 | 80 | 2 months/M | Not done | 3/5 (10,22) pooled tissues | | | TgDgCo9 | I(3),III(3),III(3),III (3),III (3) | III (2),I (2),III (2),III (2),III (2),III (2) |
| 37 | 80 | 10 years/F | Not done | 5/5 pooled tissues | | | TgDgCo10 | I (5),I (5),III (5),I (5),III (5) | II,I,III,III,II,I |
| TX 247 | | | | | | | | | |
| 15 | 160 | 15 years/M | Not done | 0/3 | 4/4 (19,19,19,31) | 0/4 | TgDgCo11 | I (4),I (4),III (4),I (4),III (4) | II (2),I (2),I (2), u-3 (2),II (2),I (2) |
| 28 | 320 | 4 months/M | Not done | 3/4 (19,19,23) | 4/4 (17,17,19,19) | 4/4 (14,17,17,17) | TgDgCo12 | I (11),III (11),III (11),III (11),III (10) | III (2),I (2),III (2),III (2),III (2),III (2) |
| 46 | 80 | 3 months/M | Not done | 1/4 | 4/4 | 4/4 (13,17) | TgDgCo13 | I (9),I (9),I (9),III (8),I (6) | I (2),III (2),III(2),I(2),I(2),III(2) |
| TX 249 | | | | | | | | | |
| 10 | 160 | 10 years/F | Not done | 0/4 | 1/4 | 1/4 | TgDgCo14 | I,III,III,I,III | II,I,III,III,III,I |
| TX 250 | | | | | | | | | |
| 15 | 80 | 10 years/M | Not done | 0/4 | 3/4(18,18,29) | 0/4 | TgDgCo15 | I (3),III (3),III (3),III (3),III (3) | III (2),I (2),III (2),III (2),III (2),III (2) |
| 33 | 160 | 5 years/F | Not done | 0/4 | 4/4 | 0/4 | TgDgCo16 | I (4),III (3),III (4),I (4),III (4) | II (2),I (2),III(2),III(2),III (2),I(2) |
| 38 | 320 | 18 years/F | Not done | 0/4 | 4/4(15) | 4/4 (10,13,15,15) | TgDgCo17 | I (8),III (8),III (8),I (8),I (8) | I (2),III (2),I (2),I (2),III (2),III (2) |
| 41 | 2560 | 5 years/F | Not done | 4/4 (16,16,16,16,) | 4/4 (14,14,14,15) | 4/4(8,13,1314) | TgDgCo18 | I (12),III (12),III (12),III (12),III (12) | III (2),I (2),III (2),III (2),III (2),III (2) |
| 43 | 80 | 2 years/M | Not done | 0/4 | 0/4 | 4/4 (14,14) | TgDgCo19 | I (4),I (4),III (4),I (4),III (4) | II (2),I (2),III (2), u-1 (2),II (2),I (2) |
| TX 254 | | | | | | | | | |
| 5 | 160 | 8 years/F | Not done | 2/4 | 4/4 (13) | 4/4 (13,17) | TgDgCo20 | I (8),I (8),III (8),I (8),III (8) | II (2),I (2),III (2),III (2),II (2),I (2) |

[a] No. of mice infected with *T. gondii*/no. of mice inoculated with canine tissues.
[b] Day of death of individual mice.
[c] Genetic analysis based on DNA obtained from no. of mice indicated.
[d] Genetic analysis based on mice inoculated with oocysts of that *T. gondii* isolate.

*gondii* oocysts 3–14 days post-ingesting canine tissues. Fecal floats were incubated in 2% sulfuric acid for 1 week at room temperature on a shaker to allow sporulation of oocysts and were bioassayed orally in mice (Dubey and Beattie, 1988). Four to 7 days after feeding oocysts, mesenteric lymph nodes of mice that died or killed were removed and after ascertaining the presence of tachyzoites, homogenates of lymph nodes were inoculated into new mice to exclude *Hammondia hammondi* infection (Dubey and Beattie, 1988). Imprints of lungs or brains of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 40–42 post-inoculation (p.i.) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 6 week post-inoculation and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

### 2.4. Genetic characterization

*T. gondii* DNA was extracted from the tissues of all infected mice from each group and strain typing was initially performed using PCR-RFLP genetic markers SAG1, SAG2, SAG3, BTUB and GRA6 (Dubey et al., 2006a). In brief, the target DNA sequences were amplified by multiplex PCR using external primers for all five markers. Multiplex PCR amplified products were then used for nested PCR with internal primers for each marker separately. Nested PCR products were treated with restriction enzymes and resolved in agarose gel by electrophoresis to reveal the RFLP patterns of the isolates. These five markers allow us to quickly characterize all samples and to identify potential mixed infection in dogs. One or two representative DNA extracts from mice infected with the same dog sample were genotyped with six additional genetic markers including c22-8, c29-2, L358, PK1, a new SAG2 and Apico to further identify isolates with high resolution (Dubey et al., in press; Su et al., 2006) by the same method described above. Allele types for all isolates were determined based on the RFLP patterns of six reference strains including RH88, PTG, CTG, COUGAR, MAS and TgCatBr5 (Su et al., 2006). These reference strains allow us to capture all known alleles for each marker and to identify potential unique alleles in new samples.

## 3. Results

Antibodies to *T. gondii* were found in 52 (16.8%) of 309 dogs with titers of 1:20 in 20, 1:40 in six, 1:80 in 17, 1:160 in three, 1:320 in three, 1:1280 or higher in three dogs.

*T. gondii* was isolated from a total of 20 dogs. The isolation rate increased with the antibody titer in the dog; the parasite was not isolated from any of 11 dogs with titers of 1:20 and any of the six dogs with titer of 1:40, but was isolated from 20 of 26 (76.9%) of dogs with titers of 1:80 or higher.

Table 3
Summary of genotyping of *T. gondii* from dogs from Bogotá, Colombia

| Genotype | Genetic markers | | | | | | | | | | | Isolate ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SAG1 | SAG2 | SAG3 | BTUB | GRA6 | c22-8 | c29-2 | L358 | PK1 | New SAG2 | Apico | |
| Reference | I | I | I | I | I | I | I | I | I | I | I | RH88 |
| Reference | II or III[a] | II | II | II | II | II | II | II | II | II | II | PTG |
| Reference | II or III | III | III | III | III | III | III | III | III | III | III | CTG |
| Reference | I | II | III | II | II | II | u-1 | I | u-2 | II | I | COUGAR |
| Reference | u-1 | I | III | III | III | u-1 | I | I | III | II | I | MAS |
| Reference | I | III | III | III | III | I | I | I | u-1 | III | I | TgCatBr5 |
| 1 | I | I | III | I | III | II | I | III | III | II | I | TgDgCo1, 2, 10, 20 |
| 2 | I | I | III | I | III | II | I | III | III | I | I | TgDgCo3 |
| 3 | I | I | III | I | III | II | I | III | u-1 | II | I | TgDgCo19 |
| 4 | I | I | III | I | III | II | I | I | u-3 | II | I | TgDgCo5, 6, 11 |
| 5 | I | I | I | III | I | I | III | III | I | I | III | TgDgCo13 |
| 6 | I | III | III | I | III | II | I | III | III | III | I | TgDgCo8, 14, 16 |
| 7 | I | III | III | I | I | I | III | I | I | III | III | TgDgCo17 |
| 8 | I | III | III | III | III | III | I | III | III | III | III | TgDgCo9, 12, 15, 18 |
| 9 | I | III | III | III | III | I | III | III | I | III | III | TgDgCo7 |
| 10 | u-1 | II | III | III | II | II | III | II | II | II | I | TgDgCo4 |

[a] At SAG1 locus, Types II and III are indistinguishable.

In total, *T. gondii* was isolated from tissues of 20 dogs (16 by bioassays in mice, 3 by bioassay in cats and 1 by bioassay in mice and cat) (Table 2). All infected mice from 7 of 17 isolates bioassayed in this host died of toxoplasmosis during primary infection. Only 10 of the 20 dogs whose tissues were bioassayed separately induced infections in mice. Interestingly, dog organs varied in their capacity to induce *T. gondii* infection in mice, hearts and tongues producing more positive results than brain (brains, hearts and tongues of four, hearts and tongues of three, heart alone of one, brain alone of one dog, and tongue alone of one dog).

The *T. gondii* isolates were designated as TgDgCo1-20 (Table 2). Genetic typing revealed 10 genotypes (Tables 2 and 3). Six genotypes (genotype #2, 3, 5, 7, 9 and 10) have one isolate only, while the rest four genotypes (genotype #1, 4, 6 and 8) have three or four isolates each (Table 3). Nine of the 10 genotypes have different combinations of known alleles at each locus, except genotype #4 that contains a new allele denoted u-3 at PK1 locus in three isolates (TgDgCo5, 6, 11). Genotypes #1 and #2 differ only at locus SAG2 (new) and genotypes #3 and #4 differ at two loci including L358 and PK1, suggesting individual genotypes in each pair are genetically closely related.

## 4. Discussion

*T. gondii* isolates differ markedly in their virulence to outbred mice. Isolates of *T. gondii* were considered virulent when mice inoculated with tachyzoites or bradyzoites died within 28 days p.i. Virulence to mice depends on several factors including the stage of the parasite, route, dose, types of mice used, host and the strain of the parasite. Among the three infectious stages of *T. gondii*, oocysts are more virulent than tachyzoites or bradyzoites for non-feline hosts (Dubey and Beattie, 1988). However, oocysts are not pathogenic for cats (Dubey, 2006). Results of our previous studies indicated that mouse virulent strains are present in apparently clinically normal chickens (Dubey et al., 2002). Results of the present study indicated that *T. gondii* isolates virulent for mice were present in apparently healthy dogs from Colombia. Therefore, *T. gondii* virulence phenotype in mouse is unique and it does not necessarily reflect the parasite's virulence in other animals. Circumstantial evidence suggests that certain genetic types of *T. gondii* may be associated with clinical ocular toxoplasmosis in humans (Khan et al., 2005, 2006). It has been suggested that Type I isolates or recombinants of Types I and III are more likely to result in clinical toxoplasmosis (Khan et al., 2005, 2006), but genetic characterization has been limited essentially to isolates from patients ill with toxoplasmosis. There is very little information regarding the genetic diversity of *T. gondii* isolates circulating in the general human population. Therefore, we must be cautious in claiming a linkage between parasite genotypes and disease presentations without the good knowledge of the *T. gondii* genotypes in human population and the environment.

In the present study *T. gondii* was isolated from tissues of 20 of 26 (76.9%) seropositive dogs with MAT titers of 1:80 or higher. Little is known of the tissue distribution of *T. gondii* in dogs. *T. gondii* is considered to have an affinity for encystment in neural tissue but this assumption is based on infections in mice. In the present study, *T. gondii* was isolated more frequently from the heart than from the brains of dogs using an equal amount of tissue for bioassay in mice. Although data on the tissue distribution of *T. gondii* are based on only 10 dogs, the results indicate that muscle tissue should be included for bioassay of canine tissues for *T. gondii* infection.

In the present study, *T. gondii* antibodies were found in 16.8% of apparently clinical normal dogs and viable parasite was isolated from 46.5% of seropositive dogs. These data are not too different from those reported by others from Brazil. Brandão et al. (2006) isolated *T. gondii* from the diaphragms of 8 of 25 dogs with leishmaniasis from Belo Horizonte, Minas Gerais, Brazil. These isolates were designated as D1 to D8; two of these eight isolates were considered to be highly pathogenic for mice. These eight isolates were previously included in molecular studies reported by their associates (Ferreira et al., 2004, 2006; Khan et al., 2006). da Silva et al. (2005) bioassayed brains of 34 of the 111 dogs with neurological signs admitted to a hospital in Paraná, Brazil. Antibodies to *T. gondii* were found in 25 of the 111 dogs in 1:16 dilution of serum tested by an indirect fluorescent antibody test (IFAT). Viable *T. gondii* was found in 36 of 36 mice inoculated with brains of nine dogs with IFAT titers of 1:64 or higher but not from two dogs with titers of 1:16 (da Silva et al., 2005). Another noteworthy observation was that all nine isolates were highly pathogenic to mice, all 36 inoculated mice died of acute toxoplasmosis 5–20 days p.i., with demonstrable tachyzoites in peritoneal exudates of mice inoculated subcutaneously with canine tissues (da Silva et al., 2005). In our study, seven of the isolates killed all inoculated mice indicating that asymptomatic dogs can harbor mouse virulent *T. gondii*.

Genotyping of 20 isolates obtained in this study revealed 10 genotypes (Table 3). A new allele denoted u-3 was identified at PK1 locus in three isolates (TgDgCo5, 6, 11). This makes PK1 the most variable locus of all 11 markers used here, with a total of 6 different alleles (I, II, III, u-1, u-2 and u-3). The results in this study, together with previous findings, indicate the high diversity of *T. gondii* strains in Colombia (Dubey et al., 2005, 2006b). Our recent study on *T. gondii* isolates from chickens in two states of Brazil also showed highly diverse and distinct parasite populations (Dubey et al., in press). All these results suggest that the tropical region in South America is a unique niche for the evolution of *T. gondii*. Further studies at the DNA sequencing level is needed to determine if the high diversity is maintained through frequent genetic crosses among a few allelic lineages or from a large effective population size or both.

## Acknowledgements

We thank Katherine Hopkins for technical assistance and the staff of the Centro de Zoonosis in Bogotá, for procuring dogs in Bogotá.

## References

Ajzenberg, D., Bañuls, A.L., Su, C., Dumètre, A., Demar, M., Carme, B., Dardé, M.L., 2004. Genetic diversity, clonality and sexuality in *Toxoplasma gondii*. Int. J. Parasitol. 34, 1185–1196.

Brandão, G.P., Ferreira, A.M., Melo, M.N., Vitor, R.W.A., 2006. Characterization of *Toxoplasma gondii* from domestic animals from Minas Gerais, Brazil. Parasite 13, 143–149.

da Silva, A.V., Pezerico, S.B., de Lima, V.Y., d'Arc Moretti, L., Pinheiro, J.P., Tanaka, E.M., Ribeiro, M.G., Langoni, H., 2005. Genotyping of *Toxoplasma gondii* strains isolated from dogs with neurological signs. Vet. Parasitol. 127, 23–27.

Dubey, J.P., 1998. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. Vet. Parasitol. 74, 75–77.

Dubey, J.P., 2006. Comparative infectivity of oocysts and bradyzoites of *Toxoplasma gondii* for intermediate (mice) and delinitive (cats) hosts. Vet. Parasitol. 140, 69–75.

Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida, pp. 1–220.

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339.

Dubey, J.P., Graham, D.H., Blackston, C.R., Lehmann, T., Gennari, S.M., Ragozo, A.M.A., Nishi, S.M., Shen, S.K., Kwok, O.C.H., Hill, D.E., Thulliez, P., 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo, Brazil: unexpected findings. Int. J. Parasitol. 32, 99–105.

Dubey, J.P., Gomez-Marin, J.E., Bedoya, A., Lora, F., Vianna, M.C.B., Hill, D., Kwok, O.C.H., Shen, S.K., Marcet, P.L., Lehmann, T., 2005. Genetic and biologic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Colombia, South America. Vet. Parasitol. 134, 67–72.

Dubey, J.P., Patitucci, A.N., Su, C., Sundar, N., Kwok, O.C.H., Shen, S.K., 2006a. Characterization of *Toxoplasma gondii* isolates in free-range chickens from Chile, South America. Vet. Parasitol. 140, 76–82.

Dubey, J.P., Su, C., Cortés, J.A., Sundar, N., Gomez-Marin, J.E., Polo, L.J., Zambrano, L., Mora, L.E., Lora, F., Jimenez, J., Kwok, O.C.H., Shen, S.K., Zhang, X., Nieto, A., Thulliez, P., 2006b. Prevalence of *Toxoplasma gondii* in cats from Colombia, South America and genetic characterization of *T. gondii* isolates. Vet. Parasitol. 141, 42–47.

Dubey, J.P., Sundar, N., Gennari, S.M., Minervino, A.H.H., Farias, N.A.R., Ruas, J.L., dos Santos, T.R.B., Cavalcante, G.T., Kwok, O.C.H., Su, C. Biologic and genetic comparison of *Toxoplasma gondii* isolates in free-range chickens from the northern Pará state and the southern state Rio Grande do Sul, Brazil revealed highly diverse and distinct parasite populations. Vet. Parasitol., in press.

Ferreira, A.M., Vitor, R.W.A., Carneiro, A.C.A.V., Brandão, G.P., Melo, M.N., 2004. Genetic viariability of Brazilian *Toxoplasma gondii* strains detected by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) and simple sequence repeat anchored-PCR (SSR-PCR). Infect. Genet. Evol. 4, 131–142.

Ferreira, A.M., Vitor, R.W.A., Gazzinelli, R.T., Melo, M.N., 2006. Genetic analysis of natural recombinant Brazilian *Toxoplasma gondii* strains by multilocus PCR-RFLP. Infect. Genet. Evol. 6, 22–31.

Howe, D.K., Sibley, L.D., 1995. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. J. Infect. Dis. 172, 1561–1566.

Howe, D.K., Honoré, S., Derouin, F., Sibley, L.D., 1997. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. J. Clin. Microbiol. 35, 1411–1414.

Khan, A., Su, C., German, M., Storch, G.A., Clifford, D.B., Sibley, L.D., 2005. Genotyping of *Toxoplasma gondii* strains from immunocompromised patients reveals high prevalence of type I strains. J. Clin. Microbiol. 43, 5881–5887.

Khan, A., Jordan, C., Muccioli, C., Vallochi, A.L., Rizzo, L.V., Belfort, R., Vitor, R.W.A., Silveira, C., Sibley, L.D., 2006. Genetic divergence of *Toxoplasma gondii* strains associated with ocular toxoplasmosis, Brazil. Emerg. Infect. Dis. 12, 942–949.

Lehmann, T., Marcet, P.L., Graham, D.H., Dahl, E.R., Dubey, J.P., 2006. Globalization and the population structure of *Toxoplasma gondii*. Proc. Natl. Acad. Sci. 103, 11423–114228.

Su, C., Zhang, X., Dubey, J.P., 2006. Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. Int. J. Parasitol. 36, 841–848.